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Art. XIII.—*The Development of Endosperm in Cereals.*

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(With 9 Text Figures.)

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The early stages of endosperm development are accurately described in the text-books and other publications dealing with the subject; but it is assumed that the process of free cell-formation, by which the first layer of endosperm arises, is continued throughout the development of the seed, and that all the endosperm cells are formed by the development of cell-walls around the nuclei that lie freely in the protoplasm of the embryo-sac. The earlier stages in the development of the endosperm of Burmannia—a monocotyledon comparable with the cereals in endosperm development—have been described by Ernst and Bernard (1). They have shown that the nuclei formed by the division of the first endosperm nucleus, do not become immediately enclosed in cell-walls, but they line the embryo-sac, and later cell-walls develop between them. From this stage Ernst and Bernard did not trace the method of endosperm development any further; they apparently took for granted that all the endosperm was formed in a similar manner.

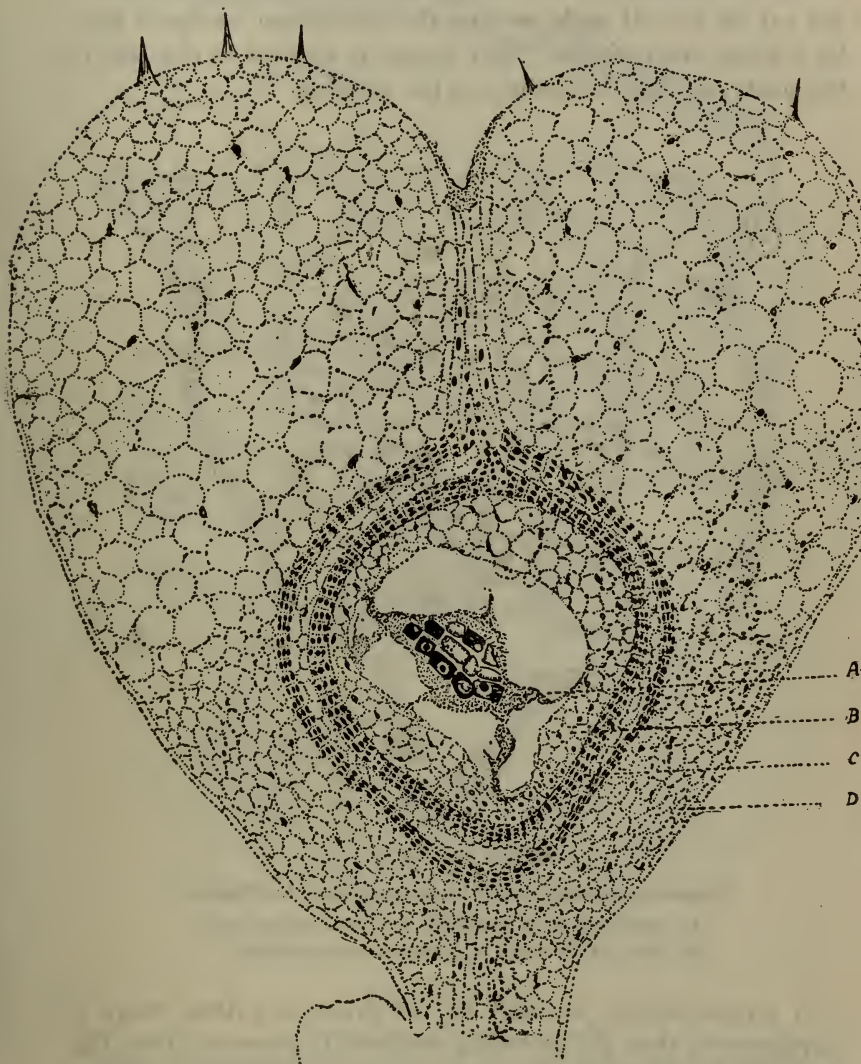
The mature grains of cereals agree in the main points of structure, having the bulk of the endosperm composed of large cells filled with starch grains, and a peripheral layer, or layers in the case of barley, containing no starch, but protein material in the form of aleurone grains. These cells also contain large nuclei, whereas the nuclei of the inner cells are much disorganised.

In a paper—The Endophytic Fungus of Lolium—published in the proceedings of the Royal Society of Victoria, Dr. McLennan (2) has described the outer layer of the endosperm of *Lolium temulentum* as an endospermic cambium, which is active only on its inner surface, where it cuts off brick-shaped cells which assume an approximately spherical form as they attain their adult size. They remain thin-walled and constitute the starchy endosperm. As the grain approaches maturity, the outer layer ceases to divide, but it persists in the seed as the aleurone layer. Even when the cells of this layer are resting, and have become filled with reserve food, their nuclei remain large and intact. At this stage the walls of the aleurone cells become considerably thickened, and this supports the cambium theory, since cambial cells entering on a period of rest show thickenings on their walls which are either partly or wholly removed when such a layer recommences its activities.

I have attempted here to trace the development of the aleurone layer and the starchy endosperm in the more common cereals, and to show whether the starchy endosperm is developed from the aleurone layer or not; that is, to prove whether the aleurone layer is really an endospermic cambium or not.

The ovules of barley, wheat and oats were taken at various stages of development, and fixed in either Carnoy's or Bouin's fixing solutions. Considerable difficulty was experienced in fixing the oat grains, owing to the hairy nature of the pericarp, which prevented the fixing solution from penetrating the seed. An attempt to fix some seeds under reduced atmospheric pressure was not any more successful, since the more volatile constituents of the fixing solutions tended to vaporise under the reduced pressure, and so pass out of the solution. The only way to ensure rapid and complete penetration of the fixing solution is to pierce the seed-coat, and even then the inner endosperm of ripe grains generally breaks in cutting. Microtome sections were cut of the grains embedded in paraffin, and the sections were stained with Haidenhain's Iron Haematoxylin as it rendered the nuclei in mitosis very distinct. In all three cases the development was found to be practically identical, except that the ripe barley grain has—as is well-known—an aleurone layer several cells deep, whereas wheat and oats have a single layer only. In the "Annals of Botany," Miss Brenchley has published two papers describing the earlier stages in the development of the grains of wheat and barley (3) and (4). They show how the first endospermic nuclei are formed by the division of the secondary nucleus of the embryo-sac, but the later stages in the endosperm development are not described.

A longitudinal section of a young ovary is shown in Text Fig. I.; it is not cut directly through the centre of the seed so that the embryo does not appear in the section. In the centre of the embryo-sac are the first-formed endospermic nuclei, which have been formed from the secondary nucleus after fertilisation. These appear as a group of free nuclei lying in the protoplasm of the embryo-sac. This, according to Goebel,



TEXT FIGURE I.

Longitudinal section of young ovary of barley after fertilisation.

A—First endospermic nuclei.
B—Nucellus.
C—Ovular integuments.
D—Wall of ovary—later pericarp.

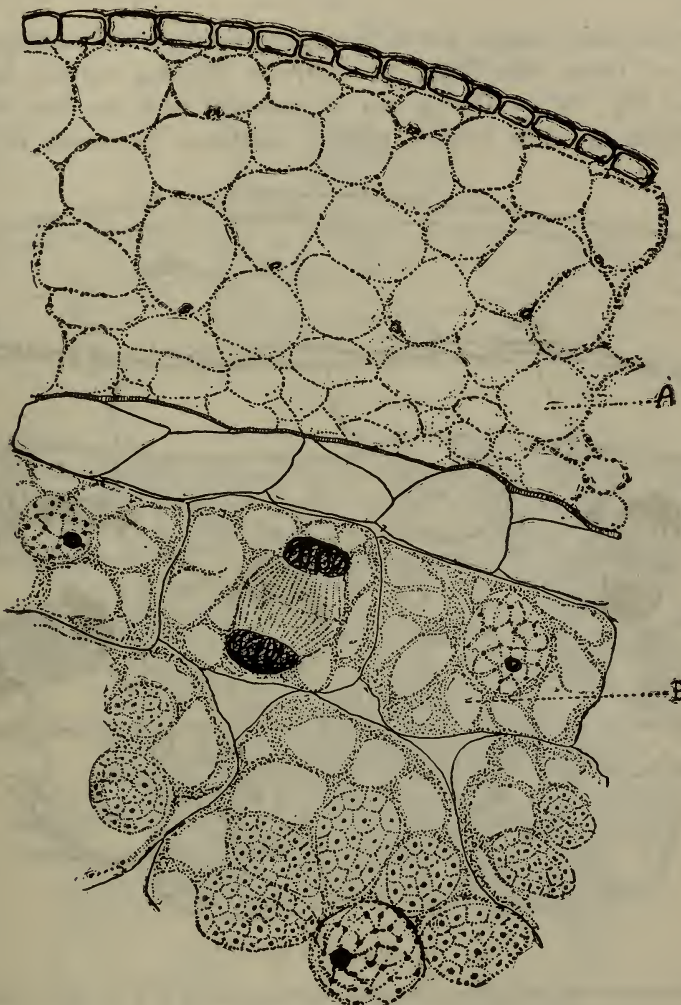
occurs in all monocotyledons and most of the dicotyledons. Some of the nuclei later pass to the walls of the embryo-sac, where they form a single lining layer (Text Fig. II.). Portions of the protoplasm around the walls, each containing a nucleus, are cut off by cell walls, so that the embryo-sac becomes lined by a single layer of cells. This section is also cut to one side of the ovary, so that the embryo is not showing.

TEXT FIGURE II.

Contents of embryo-sac of barley soon after fertilisation.

A—Group of large vacuolate nuclei in embryo-sac.
B—Wall of sac lined by protoplasm and nuclei.

A typical section through an oat grain in a later stage of development than the preceding sections is shown in Text Fig. III. One nucleus of the outer layer of endosperm cells is undergoing mitosis, and there are also two resting nuclei of the same layer apparent. After the nuclei divide, transverse cell-walls are formed between them, and the inner cells do not as a rule divide again, but they enlarge considerably and become filled with starch. The nucleus of the outer cell remains large and retains its power of dividing until the grain is almost mature. Aleurone grains then appear in the cells of the outer



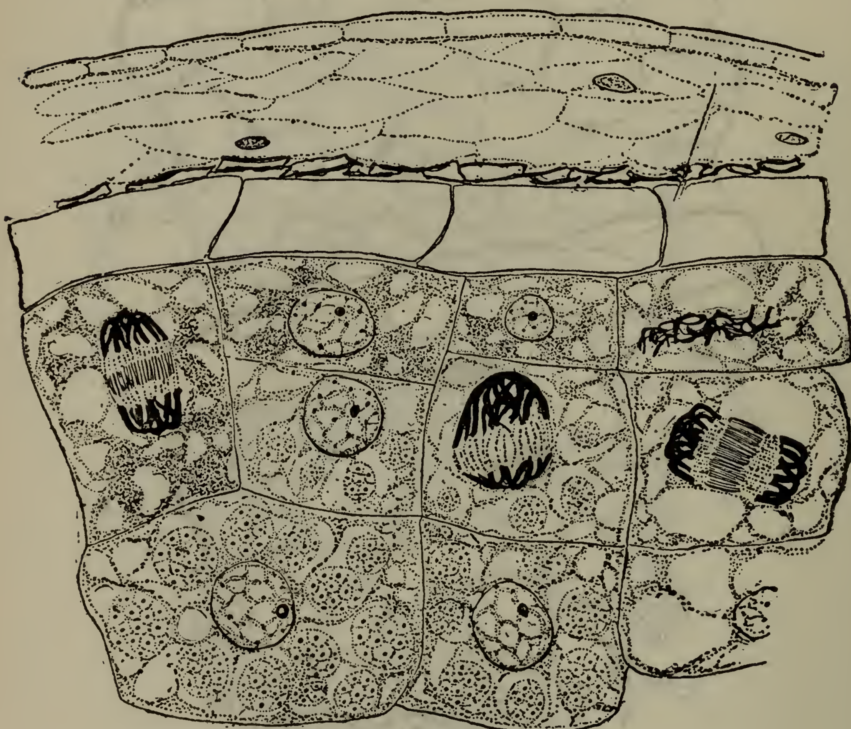
TEXT FIGURE III.

Transverse section of oat grain.

B—Endospermic cambium shows two resting nuclei and one in mitosis.
A—Degenerating cells of walls of ovary.

layer. The starch of the endosperm of oats is not in the form of grains as in wheat and barley, but appears as rounded groups. Each group is made up of a number of centres, of which there appear to be more in the older than in the younger groups; but it is possible that the number of centres is the same in all of the groups and only show up clearly when starch is deposited in them.

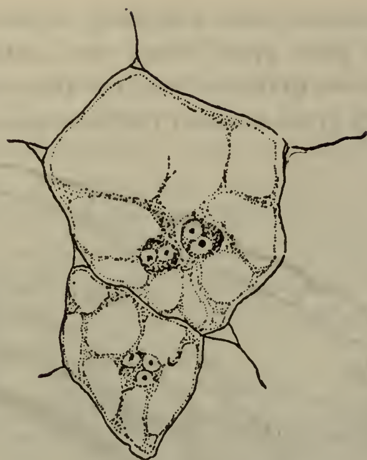
Occasionally nuclei are to be found dividing in the endosperm two or three cells below the actively dividing surface layer (Text Fig. IV.). This is not inconsistent with the idea of a cambium, as cells formed from a cambium frequently divide



Text Figure IV.

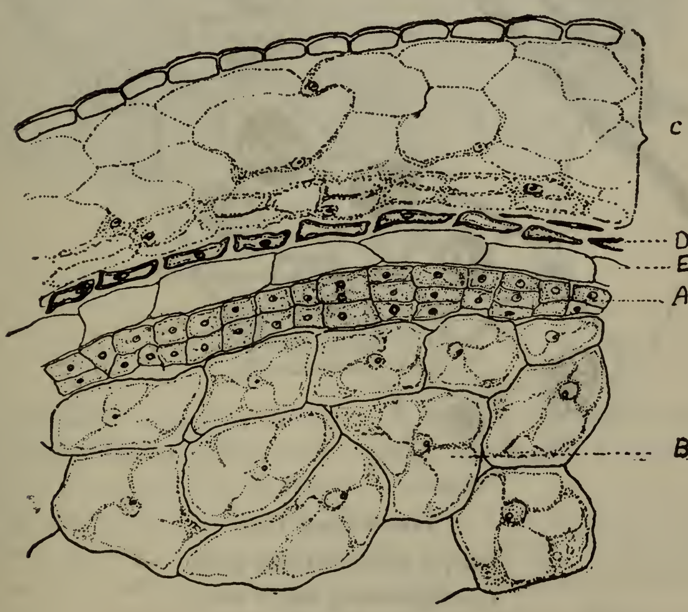
Transverse section of young oat grain showing nuclei of first and second layers in mitosis.

again. In the endosperm of barley binucleate cells sometimes occur (Text Fig. V.), and it is possible that after the original nucleus divided the two daughter-nuclei lie close together in the cell, and are prevented from separating by the presence of starch which soon becomes deposited in the cell. This also would prevent the formation of a new cell wall, thus giving a single binucleate cell.



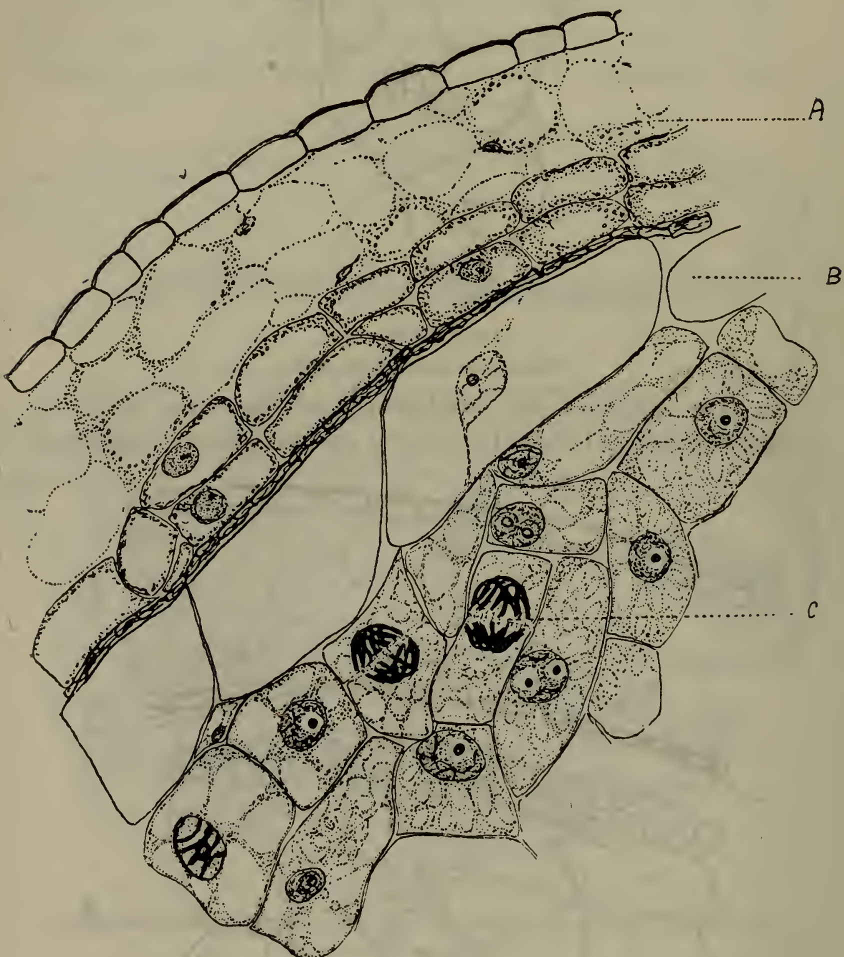
TEXT FIGURE V.
Binucleate endosperm cell.

A later stage in the development of the barley grain is illustrated by Text Fig. VI.; it shows the transition of the surface



TEXT FIGURE VI.
Transverse section of barley grain.
A— Outer dividing layers of endosperm.
B—Starchy endosperm cells.
C—Pericarp.
D—Testa.
E—Remains of nucellus.

layers of the endosperm from a dividing cambium to the resting condition of the adult grain, when the cambial cells become packed with aleurone grains to form the aleurone layer. Another section of a barley grain at about the same stage of development



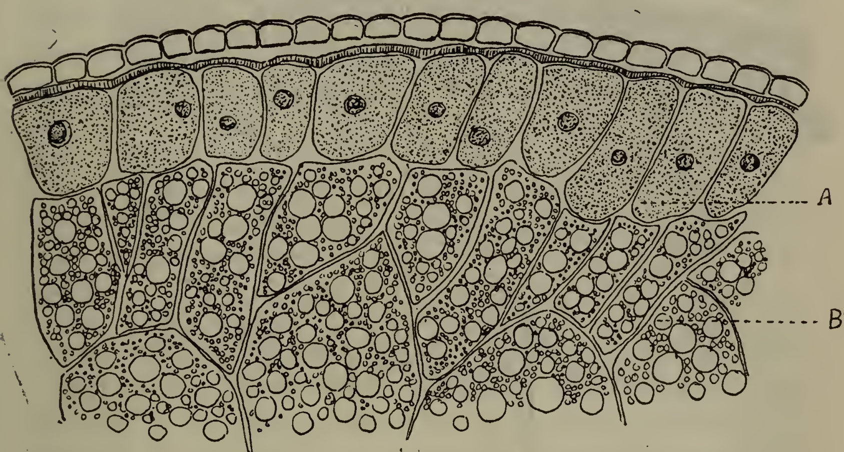
Text Figure VII.

Oblique section of barley grain.

A—Degenerating wall of ovary.
B—Remains of nucellus.
C—Dividing nucleus.

as Text Fig. VI., but which has been cut obliquely, and is more highly magnified, is shown in Text Fig. VII. The distinction between the dividing cambial layer and the general endosperm is not so well marked, but the details of cell-division are clear in the outer layer, which appears several cells deep owing to the obliqueness of the section.

Surrounding the endosperm there is a single layer of large cells, the contents of which have practically disappeared; this is the remains of the nucellus (5), which has been displaced and absorbed by the developing endosperm. It appears as a layer of disorganising cells in the young grain, but has disappeared when the grain is ripe. Beyond this there is a layer of silica, which



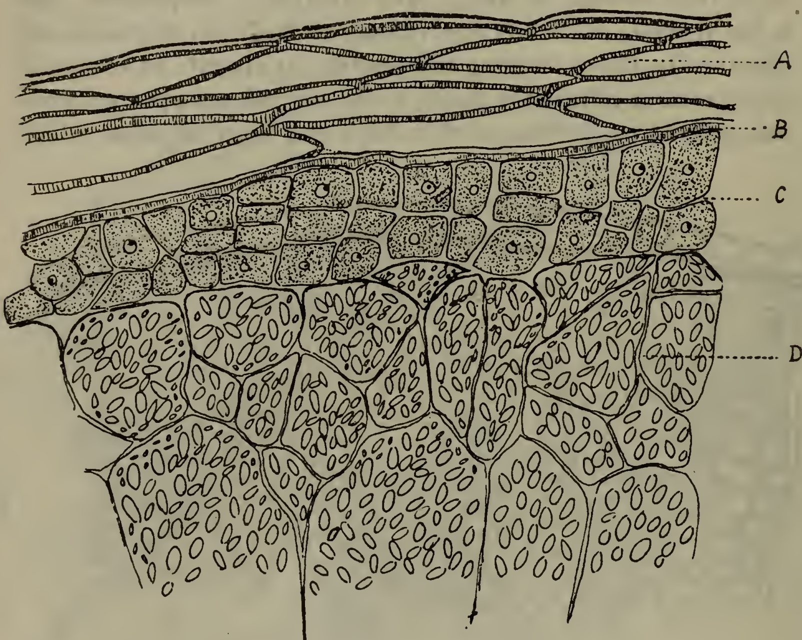
TEXT FIGURE VIII.

Transverse section of ripe oat grain.

A—Single aleurone layer.
B—Endosperm.

was probably deposited in the outer wall of the embryo-sac—this forms the so-called testa which is not really a seed-coat, since it is devoid of any cellular structure.

The ovary wall contained starch which was absorbed by the embryo during its development (Text Fig. VI). As the starch is used the cells become empty, and the nuclei can be seen in process of degeneration. Later the cell-walls thicken and develop into the pericarp of the ripe grain. The final stages, where the resting cambium appears as the aleurone layer, are shown in a transverse section of an oat grain (Text Fig. VIII.), and of a barley grain (Text Fig. IX.). With the exception of occasional cases in which dividing nuclei occur just beneath the superficial cambial layer, no cell divisions take place in the starchy portion of the endosperm, which is entirely derived from segment cells cut off from the cambium.



TEXT FIGURE IX.

Transverse section of ripe barley grain.

A—Pericarp.
B—Testa.
C—Aleurone layer several cells deep.
D—Endosperm (starch).

The fact that the aleurone layer differs from the starchy endosperm in being a resting layer, enables us to understand several of its peculiarities. Thus Steward (6) has shown that the removal of the aleurone layer from the endosperm leads to a marked fall in the output of carbon dioxide by the grain, and indicates approximately the comparatively large share of the total respiratory output that is due to the aleurone layer. Injury to the seed alone would tend to cause an increased respiratory activity manifested as a wound reaction. Although the cells of this layer are packed with aleurone grains, they also contain large, well-defined nuclei and a quantity of protoplasm. The nuclei of the starch-containing cells of the endosperm are partly disorganised and the cells contain very little protoplasm. The greater respiratory activity of the aleurone layer may be accounted for if it is to be regarded as a resting cambial layer.

It has also been demonstrated that there is present in the outer layers of the endosperm of wheat, rice and other cereals, a substance the deficiency of which causes polyneuritis in birds and Beri-Beri in man (7). Funk gave to this substance the name vitamine, and supposed it to be contained in cells rich in protein. It is found in the tissue of the embryo of cereals, as well as in the aleurone layer, but it is deficient in the starchy endosperm. Since vitamines are usually especially associated with growing tissues, it is not surprising to find that in this layer—a resting cambial layer—they should be especially abundant, whereas in endosperm cells derived from it, which degenerate into starchy receptacles, vitamines should be deficient or absent.

Brown and Morris (8) advanced the view, from their experiments, that the amylaceous endosperm of Granineae represented a "dead storehouse of reserve material." This conclusion does not refer to the endosperm as a whole, but only to the amyliferous cells, the possibility of the aleurone cells possessing vitality being left open. Haberlandt (9) regarded the aleurone layer as a glandular digestive organ.

The following table, taken from the report of a committee of the Royal Society of London, indicates that there are vitamines present in the whole grain of the cereals, but the flour which is obtained after milling contains no vitamines, as they are removed with the aleurone layer.

Vitamines in Plants.

Plant.	Fat-soluble A (Antirachitic)	Fat-soluble B (Antineuritic (Anti Beri-Beri)	Unstable C Antiscorbutic
Wheat and whole grain of cereals	+	+	0
White wheat flour	0	0	0
Cornflour	0	0	0
Polished rice	0	0	0
Malted barley	+	+ +	+ +

Summary.

(1) The first formed endospermic cells of cereals are derived from the secondary nucleus of the embryo-sac.

(2) The nuclei so formed pass to the walls of the embryo-sac, when they form a lining layer. Later the nuclei become enclosed by cell walls, so that the embryo-sac is lined by a single layer of cells.

(3) The lining layer of the embryo-sac assumes the character of a cambium, which produces segment cells only on its inner surface.

(4) The segment cells formed by the division of the cambial cells enlarge, remain thin-walled, and become packed with starch, to form the starchy endosperm.

(5) After the cells of the endospermic cambium have ceased to divide, they become filled with aleurone grains and the cell-walls thicken. It then forms the aleurone layer.

(6) The greater respiratory activity of the aleurone layer and the presence of vitamines in it are the natural results of its being a resting cambium.

(7) Whether it can be awakened to further activity during germination remains for future investigation.

The foregoing research was carried out in the Botanical Department of Melbourne University, under the direction of Professor Ewart, and represents the work done as Caroline Kay Scholar.

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